notwithstanding, this Reply (including the Declaration) will place the application in better condition for appeal should it be necessary.

As correctly indicated in the Office Action Summary, claims 46-70 are pending in the application. Claims 46-56 are under consideration and stand rejected.[1]

Claim rejections

Claims 46-56 have been rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Reddy and Sastry (Brain Research, 168:287-98, 1979). Claims 51 and 52 have been rejected under 35 U.S.C. § 103(a) as unpatentable over Reddy and Sastry. In the Amendment and Reply filed July 5, 2005, Applicants traversed both of these rejections. However, Applicants arguments were not accepted, allegedly for being merely arguments of counsel.

Applicants previously provided a mathematical analysis showing that methods and the inherent results taught by Reddy and Sastry were necessarily distinct from the presently claimed methods, and that Reddy and Sastry's only suggestions to modify their method would have led a skilled practitioner further from the presently claimed invention. The Office indicated that Applicant's arguments were not persuasive, because the Office asserted "Applicants reasoning and asserted formulas for the calculation of [the] size of the pieces of mammalian cerebral tissue cannot be accepted since the arguments of counsel cannot take the place of evidence in the record." Applicants respectfully submit that the reasoning and arguments presented in the Amendment and Reply filed July 5, 2005 should have been accepted, because the analysis was clearly a straightforward application of geometrical principles to the evidence on the record and the argument drew logical and legal conclusions directly from the analysis of the evidence. As such, Applicants analysis and argument was

[1] Claims 57-70 have been withdrawn from consideration as being directed to a non-elected invention.

not among those types of attorney argument that must be supported by a declaration or other separate evidence. *See* MANUAL OF PATENT EXAMINATION PROCEDURE § 716.01(c)(II).

Nevertheless, Applicants present herewith a Declaration By Inventor Under 37 C.F.R. § 1.132, attached as Exhibit A, which presents an analysis of the distinctions between the prior art and the present invention as testimonial evidence on the record and adds additional testimonial evidence explaining the non-obvious qualitative differences produced by the presently claimed methods. The Declaration contains the testimony of Dr. Maurice Israël, and accordingly must be accepted by the Office as evidence.

Dr. Israël is an expert in the field as shown by his curriculum vitae and bibliography attached to the Declaration. The biographical sketch is in French but, the bibliography is in English. Briefly, beginning in the late fifties in Paris, Dr. Israël studied sciences and medicine. He chose research and began experimental studies in the neuromuscular junctions in the laboratory of Professor R. Couteaux. He continued to pursue successful research in neurobiology from that time forward as evidenced by his bibliography. From 1973 to 2005, Dr. Israël was employed by the assignee of the application, Centre National de la Recherche Scientifique (CNRS, France) as Director of Research and at the time of the invention as Director of the Center for Cellular and Molecular Neurobiology. Dr. Israël is a founder of Faust Pharmaceuticals, which is the exclusive licensee of the invention.

Dr. Israël explains that Reddy and Sastry describe passing minced brain tissue in Krebs-Ringer bicarbonate solution ten times through nylon bolting cloth having mesh sizes of 433 μm, 264 μm, 130 μm, or 44 μm. *See* Reddy and Sastry at 289. Having been repeatedly passed through the cloth, Dr. Israël confirms that the suspension produced by the method that Reddy and Sastry describe could only contain pieces of tissue with dimensions no larger than

the mesh size. Reddy and Sastry teach using cloth having smaller pore sizes than 433 μm, but do not teach or suggest larger pore sizes.

Dr. Israël has attached Table 1 to his Declaration comparing the tissue suspension produced according to the teaching of Reddy and Sastry in 1979 with earlier work by Israël et al. published in 1976 (Israël et al., *Biochem. J.* 160, 113-115, 1976 (attached as Exhibit B)) and with the methods of the presently claimed invention. The Reddy and Sastry method, like the earlier work by Israel et al. produces synaptosomes, *i.e.*, pinched-off nerve endings, which are different quantitatively and qualitatively from the calibrated pieces of tissue produced by the methods of the presently claimed methods that have been named nuerocubes or microcubes by the present inventors.

Dr. Israël testifies that the largest possible size of tissue pieces made by the method of Reddy and Sastry can be estimated by assuming that passing the tissue through the cloth produces cubes with dimensions equal to the mesh size. The volume of a cube having as its dimension 433 μm on a side (the largest mesh size used by Reddy and Sastry) is $(0.433\ mm)^3 = 0.081\ mm^3$. More realistically, because brain tissue is soft, it would be understood that the pieces would not actually be geometric cubes. The pieces of soft tissue would have rounded edges and corners so that the maximum volume of the resulting pieces may be estimated by modeling the pieces as spheres. The volume of a sphere having a diameter as large as Reddy and Sastry's largest mesh size of 0.433 mm is given by the formula $4/3\ \pi\ r^3 = 0.042\ mm^3$. This represents a geometrical upper limit that is less than half the size of the pieces made according to the presently claimed method.

However, the differences between the suspension produced by Reddy and Sastry and the neurocubes produced by the presently claimed methods would actually be even greater than the simple geometrical analysis suggests. The actual tissue pieces produced by Reddy

and Sastry would be much smaller than the geometric maximum calculated above. Dr. Israël points out that, owing to shear forces, there is not a clear geometrical relationship between the mesh sizes and the size of the structure that is preserved when using the mesh sizes suggested by Reddy and Sastry. In Dr. Israël's original procedure, described in Israël et al., (Exhibit B), using 200 μm mesh produced 2.5μm synaptosomes from Torpedo. See the first column of Table 1 attached to the Declaration.

Reddy and Sastry teach and suggest methods that make suspensions of quantitatively much smaller pieces of tissue having qualitatively different properties than the pieces of tissue produced according to the methods of claim 46 and claims that depend from claim 46, but Reddy and Sastry do not suggest making a preparation of tissue comparable to the present invention.

Dr. Israël further explains that the methods described and claimed in the present application result in a preparation that provides substantial benefits over the suspension produced following the teaching of Reddy and Sastry. Passing brain tissue through a mesh below about 0.5 mm as described in the Reddy and Sastry reference, gives synaptosomes (*i.e,*. nerve terminals). By contrast, using mesh above 1 mm produces microcubes (or neurocubes), which were never prepared before the invention described in the present application. The mesh-filtration procedure utilized by Reddy and Sastry in 1979, was previously described by Dr. Israël in 1976 (Exhibit B). The aim of that procedure was to pinch-off nerve terminals (synaptosomes) with minimal damage by forcing the tissue through gradually smaller meshes. In his 1976 paper, he forced the tissue through meshes decreasing to 200μm and obtained synaptosomes of 2.5μm diameter from Torpedo. Reddy and Sastry repeated the procedure and added a further 130 μm mesh, because brain synaptosomes are smaller than in Torpedo, e.g. 0.5μm diameter. Synaptosomes produced using mesh are more

viable than those pinched-off by conventional homogenization procedures (Potter or Dounce), but less viable than slices obtained with a McIllwain chopper that kept the neuronal and glial networks intact.

Dr. Israël realized that in order to get viability, one had to do the contrary of what was previously done, that is that it was necessary to avoid decreasing the mesh size. The structures found in a brain suspension after only passing the suspension through a relatively large (1 mm) mesh had not been previously analyzed. Because brain is soft, the adequate mesh was found to be a rigid nylon 1 mm square. This was exactly the contrary of what Dr. Israël, and others, had been previously doing, since the previously practice was successively decreasing the mesh size with the aim of pinching-off nerve endings. The prior methods, including those exemplified by Reddy and Sastry, destroyed the advantageous properties of the "microcubes" produced by the methods of the present invention that were missed earlier.

The Applicant's discovery permits large quantities of suspensions to be produced at enriched concentrations. The "microcubes" are as viable as a slice, but unlike slices, microcubes can be aliquoted into calibrated test samples that can be experimentally compared. In order to calibrate the "microcubes" thus obtained, they can be suspended in large volumes, for example about 1 litre or more of physiological saline. The microcubes sediment, spontaneously forming a "powder" on the bottom of the flask. The suspension looks like snow falling in a Christmas globe. Each "microcube" (about 0.5 mm on average) is a collection of many neurons glial cells and nerve terminals, in which the connections are preserved.

Dr. Israël explains that Reddy and Sastry's teaching is substantially the same as his earlier work from 1976. The difference is simply in the size of the synaptosomes. However, Dr. Israël states that isolating "microcubes" that are small and effective "neuronal networks"

with a collection of neurons, glial cells and nerve terminals, is a real surprising invention which could not be obviously deduced from the previous synaptosome preparation techniques.

Screening drugs acting on neuronal interactions is made possible by the present invention, as it had never been before. There were problems with the options available in the prior art. Synaptosomes do not contain working neural networks. Slices cannot be calibrated. Dr. Israël explains that one slice is never sufficiently equivalent to another slice to use in an assay to make a scientific comparison, but aliquots of a suspension of microcubes are comparable, permitting direct comparison of drug effects. The procedure is simple, but this had never been tried before. Until now, Dr. Israël explains that no other laboratory could measure in the supernatant above aliquots of microcubes, the effect of a drug on 5 transmitters at a time!

Dr. Israël explains that the mesh size is critical. Below 1mm you start pinching nerve terminals; above you will get large fragments, viable like a slice. In the case of brain it seemed impossible that the organization would be made of a sum of functional units. Therefore, no one analyzed the suspension after a single, 1 mm filtration, in adequate experimental conditions. To Dr. Israël and his coworker's great surprise, it is like if the brain was made of a sum of small structures, i.e., the microcubes, which were preserved by the inventive procedure, because the density of the local connections made the microcubes slightly more solid than their surroundings. No one knew that such structures existed before, no one had ever tried to concentrate and to purify microcubes, as the present Applicants did for the first time.

Dr. Israël explains that he and his coworkers did not set out to show that the microcubes represent structures that pre-exist in the brain, independently of the mechanical

procedure, as if the brain was a sum of small ganglia. However, the existence of barrels in mouse brain, or glomeruli in cerebellum, is recognized. The denser the local connectivity is, the more solid the structure will be, which is dissociated from its environment. The fact is, with the claimed methods, the Applicants can collect a suspension of microcubes, or neurocubes, which is functional for hours, releasing a cocktail of transmitters. The claimed methods of making microcubes of brain tissue, and the benefits that can be obtained from them, were not described or appreciated by Reddy and Sastry, or anyone else in the field at the time the present application was filed.

Reddy and Sastry fail to anticipate the present invention as recited in claim 46 and its dependent claims, because among other things, Reddy and Sastry fail to teach or even suggest passing pieces of tissue through at least one grid having a mesh size to produce calibrated pieces of mammalian cerebral material having a mean size between about 0.1 mm^3 and about 5 mm^3. The method of Reddy and Sastry produces pieces of tissue so much smaller than the presently claimed method that the suspension lacks the desirable properties of the preparation that is made by the claimed method.

The present invention has been shown to be both novel and unobvious by the testimony of Dr. Israël. Accordingly, withdrawal of both of the rejections - 35 U.S.C. § 102(b) and 35 U.S.C. § 103(a) - are respectfully requested.

CONCLUSION

In view of the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order. Such action is earnestly solicited.

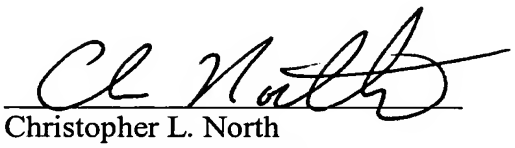
In the event that there are any questions relating to this application, it would be appreciated if the Examiner would telephone the undersigned concerning such questions so that prosecution of this application may be expedited.

The Director is hereby authorized to charge any appropriate fees that may be required by this paper, and to credit any overpayment, to Deposit Account No. 02-4800.

Respectfully submitted,

BUCHANAN INGERSOLL PC

Date: June 15, 2006

By: 

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